

COMMENT

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Exercise-induced sudden cardiac death is caused by mitochondrio-nuclear translocation of AIF

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Irrespective of the underlying risk factors, myocyte cell death is a common feature in cardiac disorders. In particular, arrhythmogenic cardiomyopathies (ACMs) are characterized by an increased incidence of apoptotic and necrotic cardiac myocytes. Since cardiac myocytes are predominantly post-mitotic¹, they are typically replaced by fibrous tissue, which further interferes with the electrophysiology of the heart, adding to the disruption of the cardiac conduction system and, thus, ACM progression. Notably, ACM patients exhibit poor prognosis and increased risk of sudden cardiac death in association with exercise. Thus, ACM is one of the rare conditions in which exercise is detrimental rather than beneficial for organismal health². However, the underlying mechanisms of this intriguing, but well-established, observation remain poorly understood. In their recent study, Chelko et al. used homozygous desmoglein-2 mutant mice (*Dsg2^{mut/mut}*), which exhibit multiple features of human ACM, to elegantly unveil the molecular underpinnings of the exercise-induced myocyte cell death in ACM³. At the core of their proposed mechanistic cascade lies the apoptosis-inducing factor (AIF), a pro-apoptotic signaling molecule that is normally located in the mitochondrial intermembrane space. However, in response to apoptotic cues, AIF moves to the nucleus, thereby triggering DNA fragmentation and cell death^{4,5}.

Chelko et al. found that almost half of *Dsg2^{mut/mut}* mice die before finishing an eleven-week protocol of endurance swimming, as compared to less than 10% in the case of WT controls. Trained *Dsg2^{mut/mut}* mice that did not die suffered from severe biventricular dilation and systolic dysfunction, and showed electrocardiographic signs of

impaired cardiac depolarization and repolarization. Concurrently, *Dsg2^{mut/mut}* hearts exhibited exaggerated fibrosis, immune cell infiltration, and increased abundance of necrotic cardiomyocytes, as determined by high-mobility-group box-1 immunostaining³. The authors went on to show that *Dsg2^{mut/mut}* hearts overexpress the Ca²⁺-activated cysteine protease calpain 1 (CAPN1), which was mainly localized at mitochondria. The cytosol-to-mitochondria translocation of CAPN1 appeared to be a prerequisite for its Ca²⁺-dependent activation. The authors also attributed such increases in CAPN1 activity to the reduced abundance of its endogenous inhibitor, calpastatin (CAST) in *Dsg2^{mut/mut}* hearts. In support of this notion, CAST overexpression or treatment with the CAPN1 inhibitor, calpeptin, delayed myocyte cell death induced by Ca²⁺ overload or CAPN1 in vitro³.

In an effort to determine the mitochondrial effectors responsible for cardiac myocyte necrosis in *Dsg2^{mut/mut}* mice, the authors examined key mediators of mitochondrial cell death. Specifically, they examined cytochrome C and apoptosis-inducing factor-1, mitochondrial (AIFM1, best known as AIF). While cytochrome C was not changed in *Dsg2^{mut/mut}* hearts, AIF exhibited significant calpain-mediated truncation (which removes a hydrophobic domain from AIF that retains it at the mitochondrial inner membrane), mitochondrio-nuclear translocation and chromatin binding in response to exercise. Importantly, Chelko et al. showed that truncated AIF undergoes nuclear translocation not only in the myocardium of exercised *Dsg2^{mut/mut}* mice, but also in ventricular samples of patients with ACM. In an interesting twist of their study, the authors demonstrated that a major proportion of cleaved AIF in *Dsg2^{mut/mut}* mice is oxidized, an effect which they attributed to exaggerated exercise-triggered oxidative stress due to an insufficient mitochondrial thioredoxin-2 ROS buffering system. In turn, the degree of AIF oxidation was found to increase its

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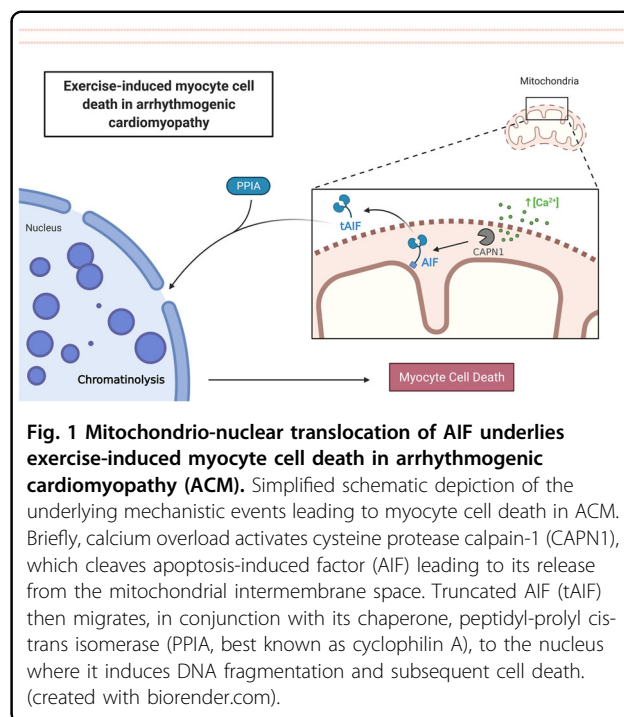


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DNA binding ability, thus facilitating AIF-mediated chromatinolysis.

Finally, in a series of exhaustive experiments the authors could show that truncated AIF causally underlies ACM-associated myocyte cell death. First, they showed that HSP70, an endogenous inhibitor of AIF, was reduced both in *Dsg2^{mut/mut}* hearts and stem cell-derived cardiomyocytes that were subjected to sustained beta-adrenergic stimulation and Ca^{2+} overload to mimic exercise. Along similar lines, both *Dsg2^{mut/mut}* myocardia and stem cell-derived cardiomyocytes exhibited higher abundance of the AIF nuclear chaperone, peptidyl-prolyl cis-trans isomerase (PPIA or cyclophilin-A), which was bound to AIF and co-migrated with it to the nucleus. Next, an AIF mimetic peptide, fused to the cell-penetrating HIV transactivator of transcription (TAT), was used to test whether blocking the interaction between AIF and PPIA would prevent cell death induced by chronic beta-adrenergic stimulation and Ca^{2+} overload in *Dsg2^{mut/mut}* stem cell-derived cardiomyocytes. AIF-TAT peptide reduced the formation and nuclear migration of PPIA–AIF complexes in these cells, thereby plummeting myocyte apoptotic death. Interestingly, AIF-TAT treatment also attenuated signs of necrotic cell death such as nuclear enlargement, loss of cardiac troponin striations, and cell membrane swelling, all of which were evident in untreated ACM myocytes. These results suggest that preventing the interaction between AIF and PPIA effectively protects from both apoptosis and necrosis.

The authors are to be commended for their diligent efforts to meticulously dissect the underlying mechanisms of exercise-induced cardiomyocyte cell death in ACM (Fig. 1). Future studies must test whether pharmacological targeting of AIF, for instance by inhibiting its oxidation, truncation, interaction with PPIA, or translocation to the nucleus, also protects against ACM-related sudden cardiac death in vivo. Thus, it will be interesting to see whether genetic inactivation of *PPIA* can correct the phenotype of *Dsg2^{mut/mut}* mice, knowing that, in *PPIA* knockout mice, reduced mitochondrio-nuclear translocation of AIF exerts neuro-protective effects against cerebral injury induced by hypoxia-ischemia⁶. It will also be interesting to examine whether cardiac non-myocytes, especially neurons and resident macrophages, which contribute to cardiac electrical conduction⁷, are subjected to similar pro-apoptotic AIF actions in response to exercise. Furthermore, future research must determine whether other AIF functions, beyond its pro-apoptotic action, are involved in ACM. For instance, AIF is implicated in the biogenesis of mitochondrial respiratory chain complexes, meaning that it is required for oxidative phosphorylation⁸. Considering the acute nature of sudden cardiac death, impaired mitochondrial function might be an added mechanism through which cleaved AIF contributes to ACM. In favor of this conjecture, Cheiko et al. reported that



Dsg2^{mut/mut} cardiac myocytes exhibit a reduced mitochondrial membrane potential, limited anti-oxidative capacity and increased oxidative stress³. Thus, it is tempting to speculate that *Dsg2^{mut/mut}* hearts might also suffer from a mitochondrial bioenergetic crisis, which might promote cardiac dysfunction and mortality upon exercise. Indeed, mitochondrial energy metabolism is increasingly implicated in various forms of cardiomyopathies, including heart failure^{9,10}. Thus, future studies should examine whether improving mitochondrial function improves ACM and reduces exercise-induced sudden death of ACM patients. Indeed, mounting evidence suggests that reduced mitochondrial ROS and Ca^{2+} accumulation^{11,12}, as well as the promotion of mitochondrial quality control mechanisms, like autophagy/mitophagy, are associated with salutary cardiac effects and protection against a wide range of cardiac diseases^{13–15}. Finally, although the study by Chelko et al. provides initial evidence for a role of AIF in human ACM, further clinical studies will be needed to corroborate the reported mechanisms in humans. To achieve this goal, larger patient cohorts with pre-mortem diagnosis of ACM, and detailed history on the cause of death and exercise training will be necessary. For this, cardiac samples, preferably collected prospectively from live ACM patient (rather than at autopsy), would be of great utility for further elucidating the pathogenesis of the disease.

In conclusion, the findings reported by Chelko et al. establish the CAPN1-PPIA-AIF axis as a firm therapeutic target candidate for treating ACM and for avoiding exercise-induced sudden cardiac death.

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Author contributions

M.A. and G.K. wrote the manuscript and prepared the figure.

Funding

M.A. acknowledges support from the European Society of Cardiology and the Austrian Society of Cardiology.

Conflict of interest

The authors declare no competing interests.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 12 March 2021 Revised: 24 March 2021 Accepted: 24 March 2021

Published online: 09 April 2021

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